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Purinergic P2X Receptors and Heightened Exercise Pressor Reflex in Peripheral Artery Disease

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Abstract

Arterial blood pressure (BP) and vasoconstriction regulated by sympathetic nerve activity (SNA) are heightened during exercise in patients with peripheral artery disease (PAD). The exercise pressor reflex is considered as a neural mechanism responsible for the exaggerated autonomic responses to exercise in PAD. A series of studies have employed a rat model of PAD to examine signal pathways at receptor and cellular levels by which the exercise pressor reflex is amplified. This review will summarize results obtained from recent human and animal studies with respect to contribution of muscle afferents to augmented SNA and BP responses in PAD. The role played by adenosine triphosphate (ATP) and ATP sensitive purinergic P2X receptors will be emphasized.

Keywords

sympathetic nerve activity; blood pressure; static exercise; muscle temperature; peripheral artery disease; P2X; bradykinin; nerve growth factor

1. Introduction

The Exercise Pressor Reflex

During exercise, sympathetic nervous activity (SNA) increases and this leads to rises in blood pressure (BP) and heart rate (HR), myocardial contractility and peripheral vasoconstriction [1, 2]. A basic mechanism termed the “Exercise Pressor Reflex” [3–6] is thought to contribute to sympathetic engagement during exercise. This autonomic reflex is initiated as thin fiber afferents arising from contracting skeletal muscle are engaged [4–6]. This system responds to mechanical deformation of the muscle afferents receptive field as well as to muscle metabolic by-products [4]. Group III afferents are predominantly mechanically sensitive (mechanoreceptor) and Group IV afferents are predominantly metabosensitive (metaboreceptor) [7]. When these receptors are stimulated, thin fiber muscle afferent nerves are engaged, cardiovascular nuclei in the brainstem are activated, SNA increases and BP and HR rise [6].

In humans, it is well recognized that the stimulation of mechanically and chemically sensitive afferents from the exercising muscles evokes significant increases in muscle

sympathetic nerve activity (MSNA) and BP [1, 8]. The role of muscle mechanoreceptors in evoking MSNA activation in humans has been further verified with low-level repeated exercise protocols [9] or repeated passive stretch [10]. In addition, the sympathetic and cardiovascular responses to exercise are modulated by the “Central Command” [11–13] and by the arterial baroreflex [14, 15].

The Exercise Pressor Reflex in Peripheral Artery Disease (PAD)

This reflex mechanism found in healthy individuals is altered in cardiovascular diseases in the processing of muscle afferent signals via afferent nerves’ receptors [16–23]. For example, as the exercise pressor reflex is activated in patients with PAD, BP and HR are exaggerated [24, 25]. These studies showed that the BP rises during walking in the PAD patients are significantly greater than that seen in healthy control subjects [24, 25]. Moreover, the BP response during the exercise with the “diseased” limb is greater than that during the exercise with the “non-diseased” limb [26]. A recent human study further indicates that the SNA driven exercise pressor reflex is accentuated in PAD [27]. Specifically, a large increase in BP within 20 second of plantar flexion exercise at a light workload is observed in PAD patients, and the magnitude of this early BP response is inversely related to the ankle–brachial index, an index of the prognosis in PAD. This early BP response occurs before claudication is noted by the subjects, which suggests that the accentuated BP response is due to an augmented muscle mechanoreflex in PAD. Moreover, another recent study [28] showed that PAD patients have augmented renal vasoconstriction during plantar flexion exercise. The augmented renal vasoconstriction occurs within the first minute of exercise, which further supports the concept that muscle mechanoreflex is exaggerated in PAD patients.

As noted, PAD caused by a restriction of the blood vessels in the lower limbs is typically popular in the older adults [29–31]. The most common symptom of this disease is intermittent claudication, which frequently occurs during physical activity but is relieved promptly by rest [32]. Nevertheless, the underlying mechanisms leading to the abnormalities in limb blood flow regulated by sympathetic system remain to be determined.

2. The Mechanisms Responsible for Amplified Exercise Pressor Reflex in PAD

A rat model of femoral artery ligation that displays impaired reserve capacity of limb blood flow with exercise, has been employed to study PAD in humans [33]. In addition, in rats muscle contraction has been performed to evoke both mechano- and metabo- components of the exercise pressor reflex, and muscle stretch has been employed to activate muscle mechanoreceptor. Lactic acid and capsaicin have been injected into the arterial blood supply of the hindlimb muscles to stimulate muscle metaboreceptors. In order to examine signal pathways, several methods have been employed. Specifically, 1) the western blot analysis was used to examine the protein levels of afferent nerves’ receptors; 2) the dual immunofluorescence techniques were employed to distinguish sensory dorsal root ganglion (DRG) neurons supplying the two different phenotypes of thin fiber (C-fiber and A-fiber); 3) the whole cell patch clamp was performed to examine functions of DRG neurons’ receptors;

and 4) the osmotic minipump was used to infuse nerve growth factor (NGF) into the hindlimb muscles.

Using this model, a number of prior studies have demonstrated that the SNA and pressor responses to static muscle contraction and stimulation of muscle metabolite receptors i.e. capsaicin sensitive TRPV1, TRPA1, purinergic P2X and acid sensing ion channel subtype 3 (ASIC3) are amplified in occluded rats as compared with control rats [18, 19, 22, 23] [34]. In addition, other muscle afferents' receptors including μ -opioid and thromboxane (TP) receptor are engaged in the reflex response in processing chronic ischemia of the hindlimb muscles [35, 36]. Stimulating peripheral μ -opioid receptors using DAMGO significantly attenuates the pressor responses to static contraction in rats with 72 hours of femoral artery ligation compared with freely perfused rats [36]. Also, this study demonstrated that the inhibitory effect of DAMGO can be prevented by the injection of naloxone, an opioid blocker. In another published work, arterial injection of daltroban, a TP receptor antagonist, into the hindlimb muscles has been shown to attenuate the cardiovascular responses to static contraction and tendon stretch in 72 hours of ligated rats, suggesting that TP receptor contributes to the exercise pressor reflex in PAD [35]. In addition, recent studies showed that blocking bradykinin B2 and stimulating peripheral δ -opioid receptors contribute to the exaggerated exercise pressor reflex via mechanically sensitive group III muscle afferents in rats with femoral artery occlusion [37, 38] [39]. Blocking PGE2 EP4 attenuates the augmented BP response to static exercise observed in rats with femoral artery occlusion [40]. In this review, a main focus is the role played by adenosine triphosphate (ATP) and ATP sensitive P2X receptor in muscle afferents in regulating the exercise pressor reflex in PAD.

3. Role of ATP and P2X in Modulating the Exercise Pressor Reflex

ATP

Data have been published demonstrating that interstitial adenosine and adenine nucleotides (ATP, ADP and AMP) levels are elevated in active muscle during exercise in human subjects as well as in animals [41–44]. Prior work has been performed to examine the role played by adenosine in evoking the reflex responses of SNA and BP to exercise. For example, reports have shown that adenosine, injected in the brachial artery, evokes the muscle reflex [45, 46]. In addition, femoral artery injection of adenosine increases SNA and BP only if the purine is allowed to re-circulate to the systemic circulation [47]. In contrast, the MSNA response evoked by handgrip exercise in humans is accentuated by local forearm infusion of theophylline, an adenosine receptor antagonist [48]. Other studies reported that adenosine does not lead to increases in MSNA and BP and to evoke the muscle reflex [49, 50]. In our recent work, interesting results are observed, showing that increasing levels of adenosine in the hindlimb muscles of rats can attenuate SNA and BP responses evoked by stimulation of TRPV1 via its A2A receptor [51]. Nonetheless, it has been consistently observed that ATP is involved in the exercise pressor reflex via muscle afferents' P2X receptors.

P2X receptor

This receptor is ionotropic and mainly located on peripheral thin fiber nerves and central sensory nerves in processing numerous sensory signals. It has been reported that ATP and analogues of ATP [such as α,β -methylene ATP (α,β -me ATP)] stimulate and excite sensory afferent nerves via P2X purinoceptors on sensory nerves in animal studies [52, 53]. Specifically, it has been shown that arterial injection of α,β -me ATP stimulates ATP-sensitive P2X receptors in the hindlimb muscle and increases arterial BP [54, 55]. It has been confirmed that Group III and IV afferents are responsible for the increase in BP after arterial administration of α,β -me ATP [56]. Also, it has been demonstrated that ATP enhances cardiovascular responses induced by stimulation of muscle mechanoreceptors *via* P2X receptors [55]. In humans, previous studies have shown that muscle interstitial ATP concentrations increase with muscle contraction [41, 57]. An important human study also examined the MSNA and BP responses to handgrip exercise when P2 receptors were blocked with local infusion of pyridoxine. The results showed that MSNA response was attenuated with P2 receptors blockade (Figure 1), which supports the concept that P2 receptors contribute to the exercise pressor reflex in humans [58]. Moreover, one study from another laboratory further showed that local antagonism of P2 receptors partially attenuated MSNA in older adults with moderately elevated systolic BP [59].

Data have been published demonstrating that interstitial ATP levels are elevated in active muscle [41–44]. It is anticipated that ischemic insult of the hindlimb muscles is likely to accumulate ATP to a larger degree, and thereby greater ATP levels can upregulate P2X receptors on thin fiber afferent nerves [60] and augment the P2X mediated-SNA response. Thus, it was hypothesized that femoral artery occlusion increases P2X receptors in DRG neurons which thereby leads to the enhanced reflex responses to stimulation of P2X. Results of our work demonstrated that 24 and 72 hours of femoral artery occlusion significantly elevates the protein levels of subtype P2X3 in lumbar DRGs [19]. Twenty hours following the ligation surgery, the level of P2X3 is 1.47 fold greater in occluded rats than in control animals. Fluorescence immunohistochemistry further confirmed that femoral occlusion increased P2X3 immunoreactivity in small-diameter of DRG neurons. In addition, injection of α,β -me ATP into the arterial blood supply of the hindlimb muscles evokes greater increases in SNA and arterial BP in occluded rats than in control rats [19] and blocking P2X attenuates the exercise pressor reflex in rats with femoral occlusion [61]. These findings suggest that there is a close linkage in increased P2X3 receptors on afferent nerves and augmented sympathetic responsiveness to stimulation of muscle afferent nerves under conditions of femoral occlusion. Another study further showed that peak amplitudes of currents with activation of P2X3 receptors are amplified in DRG neurons of limbs with femoral artery occlusion as compared with peak amplitudes in DRG neurons of control limbs [62]. This prior study also showed that increased expression of P2X3 mainly appears in DRG neurons that supply with C-fiber (group IV) afferents.

The Effects of Muscle Temperature and pH on P2X

Based on the prior findings suggesting that P2X activity is temperature-dependent [63, 64], in animal studies we have examined if elevated muscle temperature would attenuate and reduced temperature would potentiate P2X effects on muscle pressor reflex [65]. Our results

demonstrated that muscle temperature affects the reflex cardiovascular responses evoked by α,β -me ATP chemical stimulation of P2X receptors on thin fiber nerves. At higher muscle temperature (39° C), α,β -me ATP induces less of a pressor response. Also, P2X enhancement of the pressor response to muscle stretch is significantly attenuated at 39° C as compared with 35 and 37° C. This result suggests that higher muscle temperature blunts P2X effect on muscle afferent nerves-mediated pressor response.

In addition, our data suggest that muscle temperature response to static contraction is attenuated in rats with conditions of tissue ischemia induce by chronic heart failure as compared with control animals [66]. The results may have important implications for understanding cardiovascular responses to exercise where muscle temperature rises. The altered P2X-mediated responsiveness at lower or higher temperatures may influence the processing of muscle afferent signals. Thus, it would be interesting to characterize P2X3 responsiveness to temperature in primary muscle afferent neurons in order to better understand the cellular mechanism for sensory processing in PAD. It would be also interesting to examine if a higher muscle temperature in occluded limbs of PAD patients or PAD rats can improve the exaggerated SNA and BP responses. We anticipate that intervention by elevating muscle temperature will attenuate the amplified exercise pressor reflex and this will increase the walking distance in PAD patients.

In animal studies, we have also evaluated the effect of pH levels on P2X activation-induced reflex BP response since it has been shown that pH can alter P2X activity [67, 68]. We found that α,β -me ATP-induced pressor response is attenuated as the muscle interstitium is made more acidic by infusion of lower pH solutions into the femoral artery [69]. The effects of lower pH solutions are, in part, mediated via ASIC [69].

The Effects of NGF on P2X

Prior studies demonstrated that femoral artery occlusion elevates the levels of NGF in the hindlimb muscles and DRG neurons of rats [70, 71]. NGF can induce expression of P2X3 receptors in the DRG neurons [72, 73]. In addition, NGF can change the neuronal phenotype such as capsaicin-insensitive sensory neurons [74], which possibly alters afferent mediated response in the processing of sensory signals. Therefore, in a series of experiments, the role for NGF in regulating those metabolically sensitive receptors in muscle afferent nerves was examined [19, 70, 75]. Also, NGF-antibody (NGF-Ab) was previously administered into the hindlimb muscles of occluded rats to neutralize effects of NGF, and then SNA and BP responses to static muscle contraction and passive tendon stretch were examined [75]. To determine effects of NGF on the reflex responses to activation of muscle metaboreceptors, SNA and BP responses to lactic acid injected into the arterial blood supply of the hindlimb muscles were further examined after infusion of NGF-Ab in the hindlimb muscles of occluded rats [75].

Infusion of NGF into the hindlimb of healthy rats through a micro-osmotic pump induces 1.39 fold increases in P2X3 protein of the DRGs of the infused leg as compared to the control leg [19]. Also, NGF infused into the hindlimb significantly enhanced the pressor response to arterial injection of α, β -me ATP. On the other hand, blocking NGF attenuated exaggeration of the reflex response induced by α, β -me ATP in occluded rats [19]. These

findings suggest that NGF is closely related to upregulation of P2X3 expression in DRG neurons and to augmentation in the SNA and BP responses to activation of P2X3 as the hindlimb vascular insufficiency occurs.

Moreover, the role of NGF in regulating the enhanced sympathetic responsiveness during stimulation of primary muscle afferent nerves was examined as the blood flow directed to hindlimb muscles is insufficient following femoral artery ligation [75]. In this set of experiments, NGF-Ab was previously injected into the hindlimb muscles to block effects of NGF that were induced by femoral occlusion. The data demonstrated that NGF neutralization significantly attenuates femoral occlusion-augmented reflex sympathetic and BP responses evoked by static contraction and lactic acid, but not by muscle stretch. Given that administration of NGF-Ab into the hindlimb muscles significantly attenuates occlusion-enhanced protein levels of ASIC3 in DRG tissues, these results suggest that NGF that is increased in sensory nerves of occluded limbs contributes to augmented reflex SNA and BP responses to stimulation of chemically, but not mechanically sensitive muscle afferent nerves [75].

The Effects of Bradykinin on P2X

A kinin is any of structurally related polypeptides such as bradykinin and kallikrein. Bradykinin produced in contracting muscle [76] plays a role in regulating the autonomic responses by stimulating and/or sensitizing muscle afferents' kinin B₂ but not B₁ receptors [77]. It was speculated that the release of bradykinin is likely to be greater in ischemic muscle than in control muscle of healthy individuals leading to upregulation of kinin B₂ receptor [38]. As a result, the increase in muscle bradykinin may sensitize afferents responding to contraction to a greater degree in PAD, and in turn, exaggerate SNA and BP responses during muscle contraction. In our prior study, we observed that intra-arterial administration of the kinin B₂ antagonist HOE-140 into the hindlimb muscle attenuates the reflex SNA response evoked by muscle stretch in rats with femoral artery occlusion to a greater degree [38], indicating that bradykinin plays a role in exaggerated SNA responsiveness in hindlimb ischemia. In addition, bradykinin facilitates SNA and BP responses evoked by stimulation of P2X in rats [78]. It is postulated that femoral occlusion increases the protein expression of B₂ and P2X3 in DRG, and thus, the effects of bradykinin on P2X mediated-SNA responsiveness are heightened in PAD.

4. Clinical Perspective

PAD affects life styles in > 20% of adults who are older than 65 years. The narrowing of blood vessels of the lower limbs, mainly due to atherosclerotic vascular disease, is a main cause of PAD. Intermittent claudication is the most common symptom of this disease and it regularly occurs during exercise/physical activity but is relieved promptly by rest. It has been shown that exercise has benefits for patients with PAD [79]. However, exercise becomes limited due to pain and fatigue associated with the disease. Moreover, the augmented pressor response to exercise in PAD may increase the risks for the end organ damages (e.g. brain and/or heart). It is well known that the sympathetic nerve system plays an important role in regulating BP and blood flow directed to skeletal muscle tissues during exercise. Thus, the

major goal of the studies performed in our lab for the last several years was to examine the role of metabolite sensitive receptors on muscle afferent nerves (neurons) in integrating the responses of SNA after limiting blood flow to hindlimb muscle, as observed in PAD. With respect to the role of P2X, summarized results are presented (Figure 2). However, it is also important to understand how the molecular signals of afferent nerves affect the symptoms observed in human PAD through the exercise pressor reflex arc. To approach to this, therefore, some experimental strategies should be addressed in the future. It may be necessary to perform the similar experiments in conscious animals corresponding to humans, by which we can better examine the effect of certain interventions that improves the abnormal exercise pressor reflex function on the claudication and/or exercise incapacity in PAD. Moreover, a restriction of the hindlimb blood flow is simply presented in a rat model of PAD used in our currently completed animal studies. Nevertheless, intermittent claudication in human PAD is generally caused by atherosclerotic vascular disease. An animal model that is more representative of human PAD still needs to be developed in the future.

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Before Pyridoxine **After Pyridoxine** **Figure 1.**

Representative tracing of integrated muscle sympathetic nerve activity (MSNA) during the isometric handgrip, post-exercise circulatory occlusion (PECO), and the passive stretch (EOW) during PECO. Multifiber recordings of MSNA were obtained with a tungsten microelectrode inserted in the peroneal nerve of a leg. Upper panel: before pyridoxine. Lower panel: after pyridoxine. Local administration of pyridoxine in the human forearm was associated with attenuated MSNA and responses to fatiguing exercise and the PECO. (From Cui J, et al., *J. Physiol.* 2011;589:685–695, with permission from *J. Physiol.*)

Figure 2.

The diagram describes the neural pathway for the exercise pressor reflex and the role played by P2X. Interstitial ATP is increased in exercising muscle and this stimulates P2X receptors on muscle afferent nerves. In peripheral artery disease (PAD), the protein expression of P2X receptors in dorsal root ganglion (DRG) is upregulated and P2X currents of DRG neurons are increased, thereby leading the exaggerated exercise pressor reflex. Note that muscle temperature can modulate BP response evoked by activation of P2X. In addition, in ischemic muscle a decrease in pH levels and increases in nerve growth factor (NGF) and bradykinin (BK) are also engaged in mediating the effects of P2X on the reflex pressor response. CNS: central nervous system; SNA: sympathetic nervous activity; BP: blood pressure.